NeoBiota 62: 81–97 (2020)
doi: 10.3897/neobiota.62.51635
http://neobiota.pensoft.net

DISCUSSION PAPER

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Identifying safe cultivars of invasive plants: six questions for risk assessment, management, and communication

Arunava Datta[1,2], Sabrina Kumschick[1,2], Sjirk Geerts[3], John R. U. Wilson[1,2]

1 *South African National Biodiversity Institute, Kirstenbosch Research Centre, Cape Town, South Africa* **2** *Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch, South Africa* **3** *Centre for Invasion Biology, Department of Conservation and Marine Sciences, Cape Peninsula University of Technology, Cape Town, South Africa*

Corresponding author: Arunava Datta (arunavadatta@gmail.com)

Academic editor: C. C. Daehler | Received 15 March 2020 | Accepted 17 July 2020 | Published 15 October 2020

Citation: Datta A, Kumschick S, Geerts S, Wilson JRU (2020) Identifying safe cultivars of invasive plants: six questions for risk assessment, management, and communication. In: Wilson JR, Bacher S, Daehler CC, Groom QJ, Kumschick S, Lockwood JL, Robinson TB, Zengeya TA, Richardson DM (Eds) Frameworks used in Invasion Science. NeoBiota 62: 81–97. https://doi.org/10.3897/neobiota.62.51635

Abstract

The regulation of biological invasions is often focussed at the species level. However, the risks posed by infra- and inter-specific entities can be significantly different from the risks posed by the corresponding species, to the extent that they should be regulated and managed differently. In particular, many ornamental plants have been the subject of long-term breeding and selection programmes, with an increasing focus on trying to develop cultivars and hybrids that are less invasive. In this paper, we frame the problem of determining the risk of invasion posed by cultivars or hybrids as a set of six questions that map on to the key components of a risk analysis, viz., risk identification, risk assessment, risk management, and risk communication. 1) Has an infra- or inter-specific entity been proposed as "safe to use" despite at least one of the corresponding species being a harmful invasive? 2) What are the trait differences between the proposed safe alternative and its corresponding invasive species? 3) Do the differences in traits translate into a difference in invasion risk that is significant for regulation? 4) Are the differences spatially and temporally stable? 5) Can the entities be distinguished from each other in practice? 6) What are the appropriate ways to communicate the risks and what can be done to manage them? For each question, we use examples to illustrate how they might be addressed focussing on plant cultivars that are purported to be safe due to sterility. We review the biological basis of sterility, methods used to generate sterile cultivars, and the methods available to confirm sterility. It is apparent that separating invasive genetic entities from less invasive,

Copyright *Arunava Datta et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

but closely related, genetic entities in a manner appropriate for regulation currently remains unfeasible in many circumstances – it is a difficult, expensive and potentially fruitless endeavour. Nonetheless, we strongly believe that an *a priori* assumption of risk should be inherited from the constituent taxa and the onus (and cost) of proof should be held by those who wish to benefit from infra- (or inter-) specific genetic entities. The six questions outlined here provide a general, science-based approach to distinguish closely-related taxa based on the invasion risks they pose.

Keywords
cultivars, hybrids, infra-specific genetic entities, invasive species, non-invasive cultivars, ornamental plants, seedless cultivars, sterility

Introduction

Invasion is a population-level phenomenon (Petit 2004; Zenni et al. 2014). Nonetheless, most regulatory policies focus implicitly or explicitly at the species-level. Consequently, the enormous variation that exists at infra-specific levels is often not considered in regulatory frameworks. The inability to recognise differences below the species rank may lead to serious underestimation or overestimation of invasion risk (Gordon et al. 2016). For example, infra-specific entities can vary in the bioclimatic niches they occupy in their invasive ranges (Thompson et al. 2011; Gotelli and Stanton-Geddes 2015), their host-specificity (Goolsby et al. 2006), and the impacts they cause (Novoa et al. 2018). Likewise, invasions by inter-specific taxa are also very important: hybridisation is one of the major impacts caused by biological invasions (Huxel 1999; Yakandawala and Yakandawala 2011). Therefore, it is vital that policy and regulation can adequately address invasion risk at levels other than the species.

These issues are particularly significant in the context of horticulture. The introduction of plants as ornamentals constitutes a major pathway for invasive plants across the globe (Bell et al. 2003; van Kleunen et al. 2018). Many of the traits that are important for horticultural purposes can also promote invasiveness (Reichard and Hamilton 1997; van Kleunen et al. 2018), for example, the formation of dense thickets, profuse flowering, high fruit set, and wide environmental tolerance (Knight et al. 2011; van Kleunen et al. 2018). In contrast, some horticulturally-desirable traits lead to reduced competitive ability; for example, variegated leaves in plants can have lower photosynthetic performance than non-variegated leaves (Gaskin and Kazmer 2009). Horticulture, therefore, creates very particular ecologically-relevant biases in infra-specific and inter-specific genetic entities. Moreover, many ornamental plants have been subjected to artificial selection and breeding programmes to enhance specific attributes of interest (Reichard and White 2001; van Kleunen et al. 2018), leading, in some cases, to high diversity of genetic entities below and above the species rank. This can have direct consequences for the likelihood of an invasion. For example, above the species rank, hybridisation between two or more species or even genera can promote genetic diversity and increase invasiveness (Culley and Hardiman 2009; Gaskin and Kazmer 2009; Klonner et al. 2017). Below the species rank, cultivars of a species can differ in traits

such as allelopathy (Alsaadawi et al. 2012; Al-Bedairy et al. 2013) and herbicide tolerance (Sterling et al. 2004). Horticulture, therefore, creates very particular ecologically-relevant biases in infra-specific and inter-specific genetic entities.

In response to the risks of biological invasions, several countries have enacted legislation to regulate the use and trade of invasive plant species. Many of these regulated species are, however, of great ornamental value, and so such regulations cause economic losses and directly impinge on individual rights (Wirth et al. 2004). Consequently, there has been pressure to either exempt particular genetic entities that are naturally "safe" or "non-invasive" or to develop cultivars that are more environmentally sustainable (Guo et al. 2004; Freyre et al. 2014).

A specific case in point is South Africa's National Environmental Management: Biodiversity Act, Alien and Invasive Species Regulations of 2014 (Department of Environmental Affairs 2014), hereafter referred to as the NEM:BA A&IS Regulations. In an attempt to balance the goal of environmental protection with those of the horticultural industry, the regulations have provision to exempt infra- or inter-specific entities. Out of 379 plant taxa listed in the 2016 revised list, sterile cultivars or hybrids are not listed for 32 taxa, spineless varieties of two cactus species are exempted, and sterile forms of *Pinus elliotti* are regulated differently from fertile forms (Department of Environmental Affairs 2016; Suppl. material 1: Table S1). While excluding sterile cultivars or hybrids is a laudable effort to reduce potential conflicts, the regulations do not provide any guidance on how this is to be implemented, and only in one case, *Duranta erecta* "Sheena's Gold", is an acceptable cultivar specifically named. The regulations also implicitly assume that sterility is a necessary and sufficient condition to reduce invasiveness and impact. However, some of the most damaging invasive plants are predominantly sterile in their invasive range [e.g. water hyacinth (*Eichhornia crassipes*); (Zhang et al. 2010)], conversely, infra- or inter-specific entities might still be fertile, but either the reduction in fertility or changes in other traits mean that they pose an acceptable level of risk. Finally, the risk-reducing trait might not be stable, and so a 'safe' cultivar could revert to an 'invasive' plant [e.g., there is some indication that the spineless non-invasive cacti exempted under the NEM:BA A&IS Regulations might readily revert to spiny invasive forms (Novoa et al. 2019)]. While the consideration of sub-specific entities has been included in a recent risk analysis framework that is being used to provide scientific recommendations for the NEM:BA A&IS Regulations (Kumschick et al. 2020), the framework does not yet include a detailed protocol for how to analyse the relative risk of infra- or inter-specific entities.

Six questions to serve as a guide to differentiate "safe" cultivars from "risky" relatives.

To clarify the issue of how to separate "safe" cultivars from "risky" relatives, we developed a set of six questions (Fig. 1). The questions are framed so they align with the general steps of a risk analysis, i.e., risk identification, risk assessment, risk management, and risk communication.

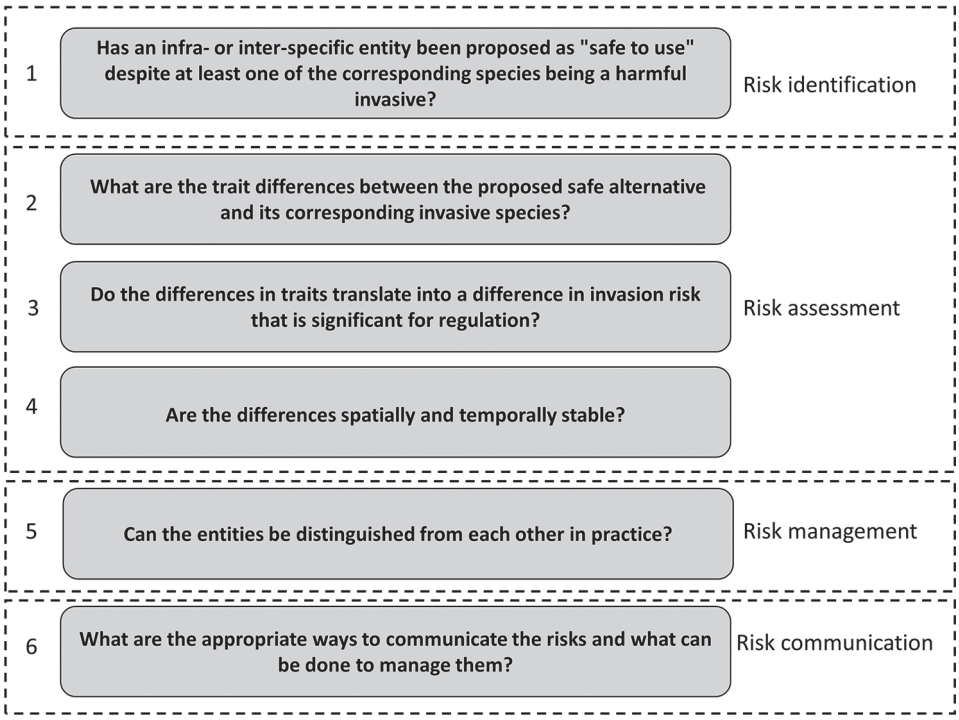

Figure 1. Six questions that should be answered if "safe" cultivars are to be differentiated from "risky" relatives in regulations on biological invasions. The questions align with the constituent parts of risk analysis as indicated by the dotted boxes. Each of the questions is explained in further details in the main text.

Question 1: Has an infra- or inter-specific entity been proposed as "safe to use" despite at least one of the corresponding species being a harmful invasive? To minimise the risk of invasion from known invasive ornamental species, the use of non-invasive and sterile forms has been promoted. Question 1 concerns identifying and specifying this problem. Is there a cultivar of an invasive ornamental species that is deemed to be safe? Is there sufficient demand for this cultivar to warrant answering the other questions? It is essential to assess the invasion risk of a supposedly non-invasive genetic entity in the context of the invasiveness of the closely-related invasive taxa or parent invasive taxa (Table 1). The list of (potentially safe) cultivars can be obtained from published cultivar names (e.g. International Cultivar Registration Authorities), nursery catalogues, and consultation with stakeholders such as plant breeders and wholesale nurseries.

Question 2: What are the trait differences between the proposed safe alternative and its corresponding invasive species? This question refers to measurable differences that could help us to characterise and differentiate between cultivars and the known invasive entity. These differences could either be due to underlying genetic differences or could be induced due to environmental factors. The traits could include vegetative traits (e.g., leaf size, presence of variegated leaves, presence of thorns and spines, height, and growth form) or reproductive traits (e.g., flower colour, phenology or number of fruits

Table 1. Selected case studies in which sterile cultivars and hybrids were specifically generated as an alternative to known invasive stocks. Details of the cultivar name, method used to generate the cultivar or hybrid, biological cause of sterility, and the commercial purpose of generating the sterile cultivar or hybrid are detailed below.

Taxa	Cultivar name(s)	Method	Cause of sterility	Purpose	Reference
Citrus	NA	Cybridisation	Cytoplasmic male sterility	Development of seedless fruits	Guo et al. (2004)
Euonymus alatus	Compactus	Ploidy alternation: Triploid plant generation	Uneven division of chromosomes	Development of sterile ornamental	Thammina et al. (2011)
Lantana camara	UF-T3 UF-T4	Interploid Hybridisation	Highly reduced pollen fertility and seed set (with seed germination highly reduced for UF-T3 and zero for UF-T4)	Development of sterile ornamental	Czarnecki et al. (2012)
Ruellia simplex	R10-102 (Mayan Purple) R10-108 (Mayan White) R12-2-1 (Mayan compact purple)	Interploid hybridisation and induced polyploidy using oryzalin	Fruitless and low pollen viability (R10-102 and R10-108), and both female and male sterility (R12-2-1)	Development of sterile ornamental	Rosanna Freyre et al. (2012)
Verbena x hybrida	SS SC	Mutation by heavy-ion beam	Non functional male and female gametes in SS and self-incompatibility in SC	To halt senescence and increase flowering duration of the plants	Kanaya et al. (2008)

or seeds). In some cases, underlying genetic differences leading to sterility may not be easily detected from phenotypic traits and, therefore, further examination of cytological and genetic differences could be necessary.

Question 3: Do the differences in traits translate into a difference in invasion risk that is significant for regulation? In question 3, we relate the observed differences (seen in question 2) to differences in the level of invasion risk posed and whether any such differences in risk mean that the taxa sit on different sides of a regulatory decision point, i.e., specimens with one set of physical properties pose an acceptable level of risk, while others do not. The observable differences in traits of the related genetic entities may lower the invasion risk only if the fecundity is directly or indirectly lower than the known invasive form. Traits that are directly related to fecundity include pollination, length of flowering time, number of flowers, fertilisation, seed production, germination success, survival rate, and vegetative reproduction. Traits that indirectly affect fecundity include allelopathic potential, mycorrhizal mutualisms, and herbivore deterrence due to the presence of thorns or chemicals. To detect differences in fecundity between different genetic entities, it is necessary to grow them in the same common garden environment and monitor long term. Ideally, the fecundity (or offspring survival) should be so low that population growth rate is negative (Knight et al. 2011).

Question 4: Are the differences spatially and temporally stable? Question 4 concerns whether the changes in the observable traits are stable and no reversal to the parental conditions is likely (see examples in Table 2). The changes should ideally be genetically fixed and not induced by environmental factors or due to short term epigenetic modifications. Even genetic changes could be reversed due to occasional outcrossing with other genotypes. Therefore, long term common garden experiments under differ-

Table 2. Selected examples of cultivar evaluation. Details of the specific method used for evaluation, number of years the evaluation took, and the main result are tabulated below. This Table corresponds to the risk assessment section (questions 2–4) of the conceptual framework proposed (Fig. 1).

Taxa	Method(s) of evaluation	Duration (Years)	Main Results	Reference
Berberis thunbergii	Common garden experiments Seed germination experiments	4–5	Out of 46 cultivars, most cultivars produced seeds. Cultivars that failed to produce seeds initially produced seed after the plants matured for 4–5 years. None of the cultivars can be considered non-invasive.	Brand et al. (2012)
Euonymus alatus	Common garden experiment Open seed germination Establishment experiment	3	None of the cultivars was completely seedless and failed to germinate. Habitat had a strong influence on seed germination and establishment.	Brand et al. (2012)
Lantana camara	Cytology Pollen staining Comment garden experiments	3	All the cultivars produced viable pollen. Almost all cultivars produced viable seeds. Even sterile triploid cultivars produced seeds when allowed to cross pollinate with diploid cultivars. None of the plants were truly sterile.	Spies and du Plessis (1987)
Nandina domestica	Common garden experiments Seed germination	1–2	Large cultivars produced more viable seeds than dwarf cultivars. Seed viability was close to zero for some cultivars which were hence recommended for use.	Knox and Wilson (2006)
Ruellia tweediana	Common garden experiment Seed germination	1–2	All the cultivars were capable of producing viable seeds that germinated. Environmental conditions (light and temperature) influenced the fecundity.	Wilson and Mecca (2003)
Spiraea japonica	Common garden experiments Pollen and seed germination Pollination experiments Flow cytometry	1	Three sterile cultivars were identified that did not produce any viable seeds and had very poor pollen germination. Sterility was not related to polyploidy.	Wilson and Hoch (2009)
Viburnum opulus	Field assessment and germination experiments.	2	All cultivars produced seeds, but varied in amount. Poor germination in open field sites compared to green house	Conklin and Sellmer (2009)

ent experimental conditions should be performed. In order to ensure that fertility does not revert, cross-pollination experiments should be performed between the different non-invasive genetic entities under consideration and the known invasive form. However, ultimately a regulator is interested in whether reversion is likely in the context of where and when (and in what numbers) the entity will be used. If a cultivar is very popular and widely planted, an extremely rare reversion is more likely to happen than for unpopular cultivars, and on-going monitoring might be advisable (see Question 6).

Question 5: Can the entities be distinguished from each other in practice? Question 5 refers to the need that, if the regulation is to be implemented, the safe cultivar must be readily distinguishable from its invasive relatives. This is particularly important for management and regulation so that non-invasive genetic entities can be exempted and monitored. Phenotypic differences might depend on growing conditions, and so other assays (Table 3) should be performed whenever necessary. Molecular markers for specific cultivars could be developed so that they can be readily detected.

Question 6: What are the appropriate ways to communicate the risks and what can be done to manage them? Finally, question 6 requires a mechanism by which recommendations are developed together with stakeholders in a transparent and inclusive manner (e.g., Novoa et al. 2018). This should be based on the results from the previous questions.

Table 3. Selected examples of cultivar identification using different techniques. In order to ensure effective regulation, the cultivar has to be distinguishable from the invasive ones. This Table corresponds to the risk management section of the conceptual framework (question 5) (Fig. 1).

Taxa	Method used	Details of the study	Reference
Castanea sativa	Pollen morphology and germination	Characterisation of sterile and fertile pollen based on pollen morphology.	Mert and Soylu (2007)
Kangaroo Paws: *Anigozanthos* and *Macropidia*	Plastid DNA sequencing	Construction of phylogenetic tree based on plastid DNA confirmed hybrid origin of invasive population and other commercially available cultivars.	Le Roux et al. (2010)
Prunus persica	Molecular markers (RAPDs)	Marker based identification of genes responsible for pollen sterility (Ps/ps).	Jun et al. (2004)
Purple-leaved Japanese barberry: *Berberis thunbergii* var. *atropurpurea* and Green leaved *Berberis thunbergii*	Shade treatments in common garden	The purple leaves of *Berberis thunbergii* var. atropurpurea become green when grown under shade. Therefore, they cannot be easily distinguished from green-leaved *Berberis thunbergii* under shaded conditions.	Lehrer and Brand (2010)

The basis of sterility and how to demonstrate it

Ultimately, the risk posed by a biological invasion is a function of population growth rate, spread rate, and subsequent impacts. Sterility in and of itself is neither a necessary nor sufficient condition to prevent damaging invasions. However, for some taxa (those that do not show asexual reproduction in particular) it is a sufficient condition and one that is particularly relevant to the development of "safe" cultivars from "risky" relatives. In this section, we review the biological bases of sterility and the different methods that have been developed to produce sterile cultivars. Furthermore, we discuss the different methods used to evaluate how "safe" a cultivar is. In each case, we highlight and discuss the links between these issues and how they address the six questions posed in Figure 1.

The biological bases of reduced fecundity and sterility

Fecundity refers to the total number of viable offspring an individual produces over a lifetime. In most plants, fecundity is measured by viable seed production. It is crucial to understand the developmental processes associated with reduced fecundity when studying invasive plants and their apparently less invasive cultivars-in the presented framework, this relates to questions 2–4. In this section, we discuss several mechanisms that can cause low fecundity in plants (viz., cytoplasmic male sterility, pollen – stigma incompatibility, developmental changes, cytological incompatibility, and abortion of embryos) and note the consequence of these for identifying "safe" cultivars.

Cytoplasmic male sterility: The inability of plants to produce functional pollen due to cytoplasmic male sterility is a well-known phenomenon across different groups of angiosperms and is attributed to cytoplasmic factors that are maternally inherited through mitochondria (Schnable and Wise 1988). Specific peptides produced in mitochondria of male-sterile plants are capable of interfering with normal pollen development. These peptides are known to reduce ATP production, enhance

the production of reactive oxygen species and cause cytotoxicity (Horn et al. 2014). Interestingly, fertility can be automatically restored (e.g. *Petunia*) in such sterile plants by the action of specific nuclear genes that express proteins which regulate the degradation of mitochondrial proteins responsible for male sterility or by affecting mitochondrial DNA organisation (Gillman et al. 2007, Horn et al. 2014). Therefore, the sterility of pollen may not be a permanent phenomenon and fertility could potentially be restored in male-sterile plants.

Pollen-stigma incompatibility: Fertilisation can occur only when a compatible type of pollen lands on the stigma. Specific proteins are known to mediate the recognition of compatible pollen with the stigmatic papillae (Mattsson et al. 1974; De Nettancourt 1997). For example, in *Brassica* self-incompatibility has been detected due to the presence of specific glycoproteins (Luu et al. 1999). In dioecy (separate male and female plants), reproductive assurance cannot be obtained through the breakdown of self-incompatibility. Interestingly, dioecious species can be as invasive as monoecious species (Daehler 1998). This could be due to leaky dioecy i.e. the ability of a dioecious species to self-fertilise by the presence of flowers of both sexes on a single plant (Venkatasamy et al. 2007). Another mechanism for incompatibility is a physiological incompatibility system that is associated with tristyly. Tristyly is a rare breeding system that ensures optimal seed production and gene flow through cross-pollination since each plant possesses only one of three tristylous morphs (Ornduff 1966). In the tristylous *Pontederia cordata* L. (Pontederiaceae), although self-incompatibility is strongest in the short-styled flowers, it can occasionally break down leading to seed formation. Interestingly, preliminary field observations throughout its invasive range in South Africa have only recorded short-styled morphed flowers and no seed production. A cultivar might appear to be infertile, but will set seed if pollinated by compatible pollen. Multiple introductions of different genotypes increase the chances of restoring fertility in such cases. This suggests that unconditional sterility can only be confirmed conducting outcrossing experiments using a wide diversity of genotypes.

Modifications of floral parts: Differentiation of floral parts is delicately orchestrated by differential gene expression. Mutations in the genes leading to interference with gene expression can lead to the formation of incomplete or defective flowers. However, interestingly, these modifications are sometimes desired traits in the horticultural industry. For example, in some cultivars of petunia, stamens are converted into an additional row of petals or sepals (van der Krol and Chua Nam Hai 1993). Although the intention behind the development of such cultivars might be purely aesthetic, they might lead to reductions in fecundity, thus potentially lowering invasion risk.

Cytogenetic anomalies: Plants can also fail to produce outcrossed seeds for cytological reasons. For example, plants with an odd level of ploidy often fail to produce viable gametes due to abnormal laggard formation during meiosis. However, apomixis can restore fecundity in such cases (Noyes 2007). *Ageratina adenophora* is an example of a highly-invasive triploid Asteraceae that can vigorously reproduce by

virtue of its apomictic seeds (Baker 1974; Noyes 2007). Additionally, some instances of successful sexual reproduction in triploid cultivars have been recorded in *Lantana camara* (Spies & du Plessis, 1987). Therefore, the use of triploid cultivars should be advocated with caution.

Abortion of fruits and seeds: is a well-known phenomenon that has been observed in a diverse group of vascular plants (Ganeshaiah and Uma Shaanker 1994; Arathi et al. 1996). Besides cytogenetic anomalies, several other genetic factors might cause abortion of embryos in seed plants. Maternal genotypes in *Pinus sylvestris* determine the seed abortion rate (Kärkkäinen et al. 1999). In *Dedeckera*, accumulation of a lethal genetic load in the populations can lead to developmental abnormalities which, in turn, lead to low viability and low germinability of seeds (Wiens et al. 1989). In the context of invasive ornamental plants, it would be desirable to grow cultivars that have inherent genetic factors that inhibit seed development rather than cultivars in which sterility has been caused by environmental cues.

Exogenous factors: Sub-optimal environmental conditions can reduce the number of seed and fruit set in plants (Lee 1988). Specific chemical triggers are also known to promote selective abortion of seeds in certain plants (Ganeshaiah and Uma Shaanker 1994). Additionally, the absence of favourable biotic interactions such as specialised pollinators in figs and orchids can lead to a seedless condition (Richardson et al. 2000). However, such exogenous factors will only limit invasiveness as long as they are in place and so might require close control if an invasion is to be prevented.

Methods to generate sterile cultivars

Many mechanisms promoting sterility or reduced fecundity discussed above can be induced or enhanced via plant breeding or molecular techniques. A wide array of such techniques to produce cultivars is currently available (see Table 1 for some case-studies). A thorough understanding of these techniques and how they induce sterility or reduce fecundity is important to understand and use questions 2–4 to distinguish between "safe" cultivars and "risky" relatives (Fig. 1).

Traditional breeding: Traditional plant breeding methods are relatively inexpensive, but they require great effort and time to screen for individuals with desired traits. Therefore, recent advances in biotechnology have been explored to produce sterile forms of invasive plants (Vining et al. 2012). Directional natural selection usually prefers the more fecund genotypes over the less fecund genotypes; as a result, the less fecund are often eliminated from the gene pool. For example, sterile triploids in nature are often lost due to natural selection. Traditional breeding methods (i.e., careful observation, artificial selection, and propagation by vegetative means) can, however, still be used to produce sterile or less fecund cultivars.

Induced polyploidy: Induction of polyploidy by the use of antimitotic agents (such as colchicine and oryzalin) has been widely used by plant breeders, as they are rela-

tively inexpensive and technically feasible. Induced polyploidy has often been used in conjunction with hybridisation techniques to produce sterile individuals (Vining et al. 2012, Freyre 2016).

Hybridisation: Hybridisation in plants may be possible between cultivars, species and even genera. Hybridisation between genetic entities with different ploidy levels often leads to sterility due to chromosomal abnormalities leading to interference with normal meiotic cell division. For example, hybridisation between hexaploid and diploid forms can result in the formation of triploid progenies which are generally sterile due to an odd ploidy level. However, in rare cases, reversal of sterility may result from cross-pollination with fertile forms (Spies and du Plessis 1987). Plants with odd chromosome numbers can also be raised from endosperm culture (Vining et al. 2012). Hybridisation experiments can, however, also potentially increase the vigour of the resulting hybrid (Ellstrand and Schierenbeck 2000), thus posing a greater risk if the sterility is accidentally reverted or if fertility is not a requirement for invasiveness. Cybridisation or somatic hybridisation is the process of producing hybrids between two sexually-incompatible individuals by fusing the protoplasm of two cells. This technique allows efficient transfer of cytoplasmic male sterility determined by mitochondrial genes (Guo et al. 2004).

Induced mutation: Mutation breeding using radiation (e.g., from x-rays, ion-beams or gamma-rays) or chemical mutagens [e.g. ethylmethanosulphonate (EMS)] is a popular technique in the toolbox of plant breeders for producing desired traits, including sterile and non-invasive forms (Broertjes and Dejong 1984; Kanaya et al. 2008).

Recombinant DNA technology: Transgenic techniques/recombinant DNA techniques can also potentially be used to transfer the genes of interest, leading to sterility (Vining et al. 2012). Such target genes could be genes responsible for cytoplasmic male sterility such as *cox2* gene (cytochrome c oxidase subunit 2) and T-urf13 gene (Štorchová 2017). However, such techniques should be used with caution, especially while working on invasive species, particularly if there is a risk of hybridisation with related native varieties or species.

Evaluation of sterile cultivars

Different methods have been used to assess the sterility of cultivars or hybrids (some key examples are listed in Table 2). These techniques range from relatively simple and easy to conduct assays (e.g, pollen staining, germination and compatibility tests, and seed viability and germination tests) to more advanced techniques relying on (e.g., molecular markers, cytological examination of chromosomes, long term common garden experiments, and pollination experiments). Here, we discuss some of these.

Pollen viability tests: Pollen staining and germination tests evaluate the quality of pollen produced by the plant. Pollen is stained with cotton blue solution and the number of viable pollen (i.e., that is stained) is counted under a microscope (Czarnecki et al. 2012). Enzymatic induction of fluorescence in viable pollen has also been used to assess the quality of pollen (Heslop-Harrison and Heslop-Harrison 1970). Pollen

germination experiments are conducted by allowing the pollen to germinate in a pollen germination media. The emergence of a pollen tube from the pollen grain is then recorded as evidence of pollen viability (Wilson and Hoch 2009).

Cytogenetic tests: Polyploidy levels can be detected by chromosomal staining during cell division or by using more recent techniques, such as flow cytometry (Wilson and Hoch 2009). Individuals with odd ploidy or an abnormal cell division process are likely to be sterile or less fecund.

Sterility genes: Molecular markers linked to genes conferring sterility can be used to screen sterile cultivars. For example, marker-based (RAPD) selection techniques have been applied to facilitate rapid identification of male-sterile cultivars of peach (Jun et al. 2004).

Common garden experiments: Common garden experiments have been used frequently to assess the fecundity of sterile cultivars. Common garden experiments are often coupled with pollination experiments to determine the stability of the sterile cultivars after outcrossing (Spies and du Plessis 1987; Lehrer et al. 2006). Although common garden experiments are crucial for any evaluation procedure, they are time-consuming, and studies confirming long-term sterility are often lacking.

Demographic models: Demographic models are used to estimate the growth rate of populations using data about various life-history stages (Easterling et al. 2000). Data collected from experiments and natural populations can be effectively coupled with demographic models, such as population matrix models and integral projection models to predict population growth rates under different scenarios (Easterling et al. 2000; Geerts 2011; Knight et al. 2011). A population with a negative growth rate might be considered safe for cultivation. However, such models and their parameterisation are often highly context-dependent and caution should be taken when extrapolating results to different habitats or climates.

Conclusions and recommendations

In this paper, we attempted to clarify the issue of distinguishing "safe" cultivars from "risky" relatives by recasting the problem as a set of six questions that align with the risk analysis process (Fig. 1). None of the individual questions is new; however, we hope that this formalisation will be valuable in providing an integrative framework for considering risks of infra- and inter- specific taxa. Although we focussed on ornamental plants, we believe that the set of questions can be extended to other situations (e.g., to breeds of animals), noting there will likely be additional ethical and cultural concerns.

While this set of six questions is, we believe, a useful formulation of the problem, answering the questions remains non-trivial. We highlighted the biological bases of reduced fecundity and sterility, and methods used to achieve and demonstrate this. However, there are many exceptions to each of the mechanisms and situations where particular methods do not work. In many cases, an unequivocal demonstration of sterility, and that any such sterility is stable, requires expensive and long-term field and molecular experiments. Various short-cut proxies of sterility have been proposed. For

example, the risk of different *Anigozanthos* spp. cultivars hybridising is a function of the ratio of their genome sizes; therefore, genetic exchange between horticultural and invasive populations can be limited if only taxa with sufficiently different genome sizes are allowed to be planted (Le Roux et al. 2010). However, long-term experiments are often necessary, specifically for woody and perennial species, before any conclusive evidence can be drawn about their invasiveness. Thus, the problem of trying to differentiate "safe" cultivars from "risky" relatives remains.

We hope the six questions outlined here will provide regulators with a basic structure around which a regulatory framework or protocol can be built and provide the horticultural industry with clarity over what needs to be demonstrated if invasions are to be avoided. However, given that the risks of invasion and impact are known from the "risky" relative, we conclude that the precautionary principle should be applied if unwanted consequences are to be avoided. We strongly believe that an *a priori* assumption of risk should be inherited from the closely-related invasive taxa from which the proposed "safe" alternatives are derived. This implies that the onus (and cost) of proof should be held by those who wish to benefit from infra- or inter-specific genetic entities.

Acknowledgements

This paper emerged from a workshop on 'Frameworks used in Invasion Science' hosted by the DSI-NRF Centre of Excellence for Invasion Biology in Stellenbosch, South Africa, 11–13 November 2019, that was supported by the National Research Foundation of South Africa and Stellenbosch University. The South African Department of Forestry, Fisheries and the Environment (DFFtE) are thanked for funding, noting that this publication does not necessarily represent the views or opinions of DFFtE or its employees. We thank Chris Daniels and Terence Mabela for discussions that helped to improve the paper.

References

Al-Bedairy NR, Alsaadawi IS, Shati RK (2013) Combining effect of allelopathic *Sorghum bicolor* L. (Moench) cultivars with planting densities on companion weeds. Archives of Agronomy and Soil Science 59: 955–961. https://doi.org/10.1080/03650340.2012.697995

Alsaadawi IS, Sarbout AK, Al-Shamma LM (2012) Differential allelopathic potential of sunflower (*Helianthus annuus* L.) genotypes on weeds and wheat (*Triticum aestivum* L.) crop. Archives of Agronomy and Soil Science 58: 1139–1148. https://doi.org/10.1080/03650340.2011.570335

Arathi HS, Ganeshaiah KN, Shaanker RU, Hegde SG (1996) Factors affecting embryo abortion in *Syzygium cuminii* (L.) Skeels (Myrtaceae). International Journal of Plant Sciences 157: 49–52. https://doi.org/10.1086/297319

Baker HG (1974) The evolution of weeds. Annual Review of Ecology and Systematics 5: 1–24. https://doi.org/10.1146/annurev.es.05.110174.000245

Brand MH, Lehrer JM, Lubell JD (2012) Fecundity of Japanese Barberry (*Berberis thunbergii*) cultivars and their ability to invade a deciduous woodland. Invasive Plant Science and Management 5: 464–476. https://doi.org/10.1614/IPSM-D-12-00029.1

Broertjes C, Dejong J (1984) Radiation induced male sterility in daisy types of *Chrysanthemum morifolium*. Euphytica 33: 433–434. https://doi.org/10.1007/BF00021141

Conklin JR, Sellmer JC (2009) Flower and seed production of Norway maple cultivars. HortTechnology 19: 91–95. https://doi.org/10.21273/HORTSCI.19.1.91

Culley TM, Hardiman NA (2009) The role of intraspecific hybridization in the evolution of invasiveness: A case study of the ornamental pear tree *Pyrus calleryana*. Biological Invasions 11: 1107–1119. https://doi.org/10.1007/s10530-008-9386-z

Czarnecki DM, Wilson SB, Knox GW, Freyre R, Deng Z (2012) UF-T3 AND UF-T4: Two sterile *Lantana camara* cultivars. HortScience 47: 132–137. https://doi.org/10.21273/HORTSCI.47.1.132

Daehler CC (1998) The taxonomic distribution of invasive angiosperm plants: Ecological insights and comparison to agricultural weeds. Biological Conservation 84: 167–180. https://doi.org/10.1016/S0006-3207(97)00096-7

David LG, Daniel SJ (1992) Self- and cross-fertilization in plants. I. Functional Dimensions. International Journal of Plant Sciences 153: 358–369. https://doi.org/10.1086/297040

Department of Environmental Affairs (2014) National Environmental Management: Biodiversity Act 2004 (Act No. 10 of 2004) Alien and Invasive Species Regulations, 2014. Government Gazette, Pretoria, 3–32 pp.

Department of Environmental Affairs (2016) National Environmental Management: Biodiversity Act 2004 (Act No. 10 of 2004) Alien and Invasive Species Lists, 2016. Vol. 864, issue 40166, 31–104.

Easterling MR, Ellner SP, Dixon PM, River S (2000) Size-specific sensitivity: Applying a new structured population model. Ecology 81: 694–708. https://doi.org/10.1890/0012-9658(2000)081[0694:SSSAAN]2.0.CO;2

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences 97(13): 7043–7050. https://doi.org/10.1073/pnas.97.13.7043

Freyre R (2016) Breeding for sterility in invasive ornamental plants. Acta Horticulturae 1140: 71–75. https://doi.org/10.17660/ActaHortic.2016.1140.13

Freyre R, Moseley A, Knox GW, Wilson SB (2012) Fruitless *Ruellia simplex* R10-102 ('Mayan Purple') and R10-108 ('Mayan White'). HortScience 47: 1808–1814. https://doi.org/10.21273/HORTSCI.47.12.1808

Freyre R, Wilson SB, Knox GW (2014) Breeding *Ruellia* and trialing for sterility at the University of Florida. 1055: 431–435. https://doi.org/10.17660/ActaHortic.2014.1055.91

Ganeshaiah KN, Uma Shaanker R (1994) Seed and fruit abortion as a process of self organization among developing sinks. Physiologia Plantarum 91: 81–89. https://doi.org/10.1111/j.1399-3054.1994.tb00662.x

Gaskin JF, Kazmer DJ (2009) Introgression between invasive saltcedars (*Tamarix chinensis* and *T. ramosissima*) in the USA. Biological Invasions 11: 1121–1130. https://doi.org/10.1007/s10530-008-9384-1

Gillman JD, Bentolila S, Hanson MR (2007) The petunia restorer of fertility protein is part of a large mitochondrial complex that interacts with transcripts of the CMS-associated locus. The Plant Journal 49: 217–227. https://doi.org/10.1111/j.1365-313X.2006.02953.x

Goolsby JA, De Barro PJ, Makinson JR, Pemberton RW, Hartley DM, Frohlich DR (2006) Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. Molecular Ecology 15: 287–297. https://doi.org/10.1111/j.1365-294X.2005.02788.x

Gordon DR, Flory SL, Lieurance D, Hulme PE, Buddenhagen C, Caton B, Champion PD, Culley TM, Daehler C, Essl F, Hill JE, Keller RP, Kohl L, Koop AL, Kumschick S, Lodge DM, Mack RN, Meyerson LA, Pallipparambil GR, Panetta FD, Porter R, Pyšek P, Quinn LD, Richardson DM, Simberloff D, Vilà M (2016) Weed risk assessments are an effective component of invasion risk management. Invasive Plant Science and Management 9: 81–83. https://doi.org/10.1614/IPSM-D-15-00053.1

Gotelli NJ, Stanton-geddes J (2015) Climate change, genetic markers and species distribution modelling. Vol 42: 1577–1585. https://doi.org/10.1111/jbi.12562

Geerts S (2011) Assembly and disassembly of bird pollination communities at the Cape of Africa (PhD thesis). Stellenbosch University, Stellenbosch.

Guo WW, Prasad D, Cheng YJ, Serrano P, Deng XX, Grosser JW (2004) Targeted cybridization in citrus: transfer of Satsuma cytoplasm to seedy cultivars for potential seedlessness. Plant Cell Reports 22: 752–758. https://doi.org/10.1007/s00299-003-0747-x

Heslop-Harrison J, Heslop-Harrison Y (1970) Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. Biotechnic and Histochemistry 45: 115–120. https://doi.org/10.3109/10520297009085351

Horn R, Gupta KJ, Colombo N (2014) Mitochondrion role in molecular basis of cytoplasmic male sterility. Mitochondrion 19: 198–205. https://doi.org/10.1016/j.mito.2014.04.004

Huxel GRR (1999) Rapid displacement of native species by invasive species: effects of hybriziation. Biological Conservation 89: 143–152. https://doi.org/10.1016/S0006-3207(98)00153-0

Jun JH, Chung KH, Jeong SB, Lee HJ (2004) An RAPD marker linked to the pollen sterility gene ps in peach (*Prunus persica*). Journal of Horticultural Science and Biotechnology 79: 587–590. https://doi.org/10.1080/14620316.2004.11511810

Kanaya T, Saito H, Hayashi Y, Fukunishi N, Ryuto H, Miyazaki K, Kusumi T, Abe T, Suzuki KI (2008) Heavy-ion beam-induced sterile mutants of verbena (*Verbena* × *hybrida*) with an improved flowering habit. Plant Biotechnology 25: 91–96. https://doi.org/10.5511/plantbiotechnology.25.91

Kärkkäinen K, Savolainen O, Koski V (1999) Why do plants abort so many developing seeds: bad offspring or bad maternal genotypes? Evolutionary Ecology 13: 305–317. https://doi.org/10.1023/A:1006746900736

van Kleunen M, Essl F, Pergl J, Brundu G, Carboni M, Dullinger S, Early R, González-Moreno P, Groom QJ, Hulme PE, Kueffer C, Kühn I, Máguas C, Maurel N, Novoa A, Parepa M, Pyšek P, Seebens H, Tanner R, Touza J, Verbrugge L, Weber E, Dawson W, Kreft H, Weigelt P, Winter M, Klonner G, Talluto M V., Dehnen-Schmutz K (2018) The changing role of ornamental horticulture in alien plant invasions. Biological Reviews 93: 1421–1437. https://doi.org/10.1111/brv.12402

Klonner G, Dullinger I, Wessely J, Bossdorf O, Carboni M, Dawson W, Essl F, Gattringer A, Haeuser E, van Kleunen M, Kreft H, Moser D, Pergl J, Pyšek P, Thuiller W, Weigelt P, Winter M, Dullinger S (2017) Will climate change increase hybridization risk between potential plant invaders and their congeners in Europe? Diversity and Distributions 23: 934–943. https://doi.org/10.1111/ddi.12578

Knight TM, Havens K, Vitt P (2011) Will the use of less fecund cultivars reduce the invasiveness of perennial plants? BioScience 61: 816–822. https://doi.org/10.1525/bio.2011.61.10.11

Knox GW, Wilson SB (2006) Evaluating North and South Florida landscape performance and fruiting of ten cultivars and a wild-type selection of *Nandina domestica*, a potentially invasive shrub. Journal of Environmental Horticulture 24: 137–142. https://doi.org/10.24266/0738-2898-24.3.137

van der Krol AR, Chua Nam Hai (1993) Flower development in Petunia. Plant Cell 5: 1195–1203. https://doi.org/10.2307/3869773

Kumschick S, Wilson JRU, Foxcroft LC (2020) A framework to support alien species regulation: the Risk Analysis for Alien Taxa (RAAT). In: Wilson JR, Bacher S, Daehler CC, Groom QJ, Kumschick S, Lockwood JL, Robinson TB, Zengeya TA, Richardson DM (Eds) Frameworks used in Invasion Science. NeoBiota 62: 213–239. https://doi.org/10.3897/neobiota.62.51031

Lee TD (1988) Patterns of fruit and seed production. In: Doust JL, Doust LL (Eds) Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, Oxford, 179–202.

Lehrer JM, Brand MH (2010) Purple-leaved Japanese Barberry (var. atropurpurea) Genotypes Become Visually Indistinguishable from Greenleaved Genotypes (*Berberis thunbergii* DC.) at Low Light Levels 28: 187–189. https://doi.org/10.24266/0738-2898-28.3.187

Lehrer JM, Brand MH, Lubell JD (2006) Four cultivars of Japanese Barberry demonstrate differential reproductive potential under landscape conditions. HortScience 41: 762–767. https://doi.org/10.21273/HORTSCI.41.3.762

Luu DT, Marty-Mazars D, Trick M, Dumas C, Heizmann P (1999) Pollen-stigma adhesion in Brassica spp involves SLG and SLR1 glycoproteins. Plant Cell 11: 251–262. https://doi.org/10.1105/tpc.11.2.251

Mattsson O, Knox R, Heslop-Harrison J, Heslop-Harrison Y (1974) Protein Pellicle of Stigmatic Papillae as a Probable Recognition Site in Incompatibility Reactions. Nature 247: 298–300. https://doi.org/10.1038/247298a0

Mert C, Soylu A (2007) Morphology and anstomy of pollen stain from male-fertile and male-sterile cultivars of chestnut(*Castabe sativa* Mill.). Journal of Horticultural Science and Biotechnology 82: 474–480. https://doi.org/10.1080/14620316.2007.11512261

De Nettancourt D (1997) Incompatibility in angiosperms. Sexual Plant Reproduction 10: 185–199. https://doi.org/10.1007/s004970050087

Novoa A, Shackleton R, Canavan S, Cybele C, Davies SJ, Dehnen-Schmutz K, Fried J, Gaertner M, Geerts S, Griffiths CL, Kaplan H (2018) A framework for engaging stakeholders on the management of alien species. Journal of Environmental Management. 205: 286–297. https://doi.org/10.1016/j.jenvman.2017.09.059

Novoa A, Flepu V, Boatwright J (2018) Is spinelessness a stable character in cactus pear cultivars? Implications for invasiveness. Journal of Arid Environments. https://doi.org/10.1016/j.jaridenv.2018.09.013

Noyes RD (2007) Apomixis in the Asteraceae: Diamonds in the Rough. Functional Plant Science and Biotechnology 1: 207–222.

Ornduff R (1966) The breeding system of *Pontederia cordata*. Bulletin of the Torrey Botanical Club 93: 407–416. https://doi.org/10.2307/2483413

Petit RJ (2004) Biological invasions at the gene level. Diversity and Distributions 10: 159–165. https://doi.org/10.1111/j.1366-9516.2004.00084.x

Reichard SH, Hamilton CW (1997) Predicting invasions of woody plants introduced into North America. Conservation Biology 11: 193–203. https://doi.org/10.1046/j.1523-1739.1997.95473.x

Reichard SH, White P (2001) Horticulture as a pathway of invasive plant introductions in the United States. BioScience 51: 103–113. https://doi.org/10.1641/0006-3568(2001)051[0103:HAAPOI]2.0.CO;2

Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmánek M (2000) Plant invasions-the role of mutualisms. Biological Reviews 75: 65–93.

Le Roux JJ, Geerts S, Ivey P, Krauss S, Richardson DM, Suda J, Wilson JRU (2010) Molecular systematics and ecology of invasive Kangaroo Paws in South Africa: Management implications for a horticulturally important genus. Biological Invasions 12: 3989–4002. https://doi.org/10.1007/s10530-010-9818-4

Schnable SP, Wise PR (1988) The molecular basis of cytoplasmic male sterility and fertility restoration. Trends in Plant Science 3: 175–180. https://doi.org/10.1016/S1360-1385(98)01235-7

Spies JJ, du Plessis H (1987) Sterile *Lantana camara*: fact or theory. South African Journal of Plant and Soil 4: 171–174. https://doi.org/10.1080/02571862.1987.10634968

Sterling TM, Thompson DC, Abbott LB (2004) Implications of invasive plant variation for weed management. Weed Technology 18: 1319–1324. https://doi.org/10.1614/0890-037X(2004)018[1319:IOIPVF]2.0.CO;2

Štorchová H (2017) The role of non-coding RNAs in cytoplasmic male sterility in flowering plants. International Journal of Molecular Sciences 18: 1–13. https://doi.org/10.3390/ijms18112429

Thammina C, He M, Lu L, Cao K, Yu H, Chen Y, Tian L, Chen J, Mcavoy R, Ellis D, Zhao D, Wang Y, Zhang X, Li Y (2011) In vitro regeneration of triploid plants of *Euonymus alatus* 'Compactus' (Burning Bush) from endosperm tissues. HortScience 46: 1141–1147. https://doi.org/10.21273/HORTSCI.46.8.1141

Thompson GD, Robertson MP, Webber BL, Richardson DM, Le Roux JJ, Wilson JRU (2011) Predicting the subspecific identity of invasive species using distribution models: *Acacia saligna* as an example. Diversity and Distributions 17: 1001–1014. https://doi.org/10.1111/j.1472-4642.2011.00820.x

Venkatasamy S, Khittoo G, Keeley S (2007) Leaky dioecy in *Diospyros* (Ebenaceae) endemic to the Island of Mauritius. Plant Ecology 189: 139–146. https://doi.org/10.1007/s11258-006-9171-y

Vining KJ, Contreras RN, Ranik M, Strauss SH (2012) Genetic methods for mitigating invasiveness of woody ornamental plants: research needs and opportunities. HortScience 47: 1210–1216. https://doi.org/10.21273/HORTSCI.47.9.1210

Wiens D, Nickrent DL, Davern CI, Calvin CL, Vivrette NJ (1989) Developmental failure and loss of reproductive capacity in the rare palaeoendemic shrub *Dedeckera eurekensis*. Nature 338: 65–67. https://doi.org/10.1038/338065a0

Wilson RL, Hoch WA (2009) Identification of sterile, noninvasive cultivars of Japanese spirea. HortScience 44: 2031–2034. https://doi.org/10.21273/HORTSCI.44.7.2031

Wilson SB, Mecca LK (2003) Seed production and germination of eight cultivars and the wild type of *Ruellia tweediana*: a potentially invasive. Journal of Environmental Horticulture 21: 137–143. https://doi.org/10.24266/0738-2898-21.3.137

Wirth FF, Davis KJ, Wilson SB (2004) Florida nursery sales and economic impacts of 14 potentially invasive landscape plant species. Journal of Environmental Horticulture 22: 12–16. https://doi.org/10.24266/0738-2898-22.1.12

Yakandawala D, Yakandawala K (2011) Hybridization between native and invasive alien plants: an overlooked threat to the biodiversity of Sri Lanka. Ceylon Journal of Science (Biological Sciences) 40(1): 13–23. https://doi.org/10.4038/cjsbs.v40i1.3403

Zenni RD, Lamy JB, Lamarque LJ, Porté AJ (2014) Adaptive evolution and phenotypic plasticity during naturalization and spread of invasive species: implications for tree invasion biology. Biological Invasions 16: 635–644. https://doi.org/10.1007/s10530-013-0607-8

Zhang YY, Zhang DY, Barrett SCH (2010) Genetic uniformity characterizes the invasive spread of water hyacinth (*Eichhornia crassipes*), a clonal aquatic plant. Molecular Ecology 19: 1774–1786. https://doi.org/10.1111/j.1365-294X.2010.04609.x

Supplementary material 1

Table S1. Plant taxa listed under South African regulations for which certain sub-specific entities are listed differently from other entities

Authors: Arunava Datta, Sabrina Kumschick, Sjirk Geerts, John R. U. Wilson

Data type: species data

Explanation note: Plant taxa listed under the South African National Environmental Management: Biodiversity Act, Alien and Invasive Species Regulations as amended in 2016, for which certain sub-specific entities are listed differently from other entities. There is no published account as to why these taxa were selected.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.62.51635.suppl1

Supplementary Table S1. Plant taxa listed under the South African National Environmental Management: Biodiversity Act, Alien and Invasive Species Regulations as amended in 2016, for which certain sub-specific entities are listed differently from other entities. There is no published account as to why these taxa were selected.

SPECIES	CATEGORY / AREA
Acer negundo L.	a. 3 b. Sterile cultivars or hybrids are not listed.
Ageratum houstonianum Mill.	a. 1b b. Sterile cultivars or hybrids are not listed.
Berberis thunbergii DC.	a. 3 b. Sterile cultivars or hybrids are not listed.
Buddleja davidii Franch.	a. 3 b. Sterile cultivars or hybrids are not listed.
Canna indica L.	a. 1b b. Sterile cultivars or hybrids are not listed.
Catharanthus roseus (L.) G.Don	a. 1b b. Sterile cultivars or hybrids are not listed.
Cestrum species not specifically listed	a. 3 b. Sterile cultivars or hybrids are not listed.
Coreopsis lanceolata L.	a. 1a b. Sterile cultivars or hybrids are not listed.
Cortaderia selloana (Schult.) Asch. & Graebn.	a. 1b b. Sterile cultivars or hybrids are not listed.
Duranta erecta L. (= *D. repens* L., *D. plumieri* Jacq.)	a. 3 in Gauteng, Kwazulu-Natal, Limpopo, Mpumalanga and North-West. b. 2 for breeding in nurseries in Gauteng, Kwazulu-Natal, Limpopo, Mpumalanga and North-West, but may not be transferred within these Provincial boundaries. c. Not listed elsewhere. d. Sterile cultivars or hybrids are not listed. e. “Sheena's Gold” cultivar is not listed.
Gleditsia triacanthos L.	a. 1b b. Sterile cultivars or hybrids are not listed.
Hedera canariensis Willd. (= *Hedera helix* L. subsp. *canariensis* (Willd.) Cout.)	a. 3 b. Sterile cultivars or hybrids are not listed.
Hedera helix L. (= *Hedera helix* L. subsp. *helix*)	a. 3 b. Sterile cultivars or hybrids are not listed.
Ipomoea indica (Burm.) Merr. (= *I. congesta* R.Br.)	a. 1b b. Sterile cultivars or hybrids are not listed.
Ipomoea purpurea (L.) Roth	a. 1b b. Sterile cultivars or hybrids are not listed.
Ligustrum lucidum W.T.Aiton	a. 1b in Eastern Cape, KwaZulu-Natal, Limpopo, Mpumalanga, North-West and Western Cape.

SPECIES	CATEGORY / AREA
	b. 3 in Free State, Gauteng and Northern Cape. c. Sterile cultivars or hybrids are not listed.
Ligustrum ovalifolium Hassk.	a. 1b in Eastern Cape, KwaZulu-Natal, Limpopo, Mpumalanga, North-West and Western Cape. b. 3 in Free State, Gauteng and Northern Cape. c. Sterile cultivars or hybrids are not listed.
Limonium sinuatum (L.) Mill.	a. 1b in Northern Cape and Western Cape. b. Not listed elsewhere. c. Sterile cultivars or hybrids are not listed.
Metrosideros excelsa Sol. ex Gaertn. (= *M. tomentosa* A.Rich.)	a. 1a in the Overstrand District. b. Not listed elsewhere. c. Sterile cultivars or hybrids are not listed.
Morus alba L.	a. 3 b. Sterile cultivars or hybrids are not listed. c. The fruit of the white mulberry is not listed if used for human consumption.
Murraya paniculata (L.) Jack. (= *M. exotica* L.)	a. 1b in KwaZulu-Natal, Limpopo and Mpumalanga. b. 2 for breeding in nurseries in KwaZulu-Natal, Limpopo and Mpumalanga, but may not be transferred within these Provincial boundaries. c. Not listed elsewhere. d. Sterile cultivars or hybrids are not listed.
Nephrolepis cordifolia (L.) C.Presl (= *Polypodium cordifolium* L.)	a. 1b in Eastern Cape, KwaZulu-Natal, Mpumalanga, Limpopo and Western Cape. b. 3 in Free State, Gauteng, North-West and Northern Cape. c. Sterile cultivars or hybrids are not listed.
Nephrolepis exaltata (L.) Schott (= *Polypodium exaltatum* L.)	a. 1b in Eastern Cape, KwaZulu-Natal, Mpumalanga, Limpopo and Western Cape. b. 3 in Free State, Gauteng, North-West and Northern Cape. c. Sterile cultivars or hybrids are not listed.
Nerium oleander L.	a. 1b b. Sterile cultivars or hybrids are not listed.
Opuntia ficus-indica (L.) Mill. (= *O. megacantha* Salm-Dyck)	a. 1b b. Spineless cactus pear cultivars and selections are not listed. c. The fruit of the sweet prickly pear is not listed if used for human consumption.
Opuntia robusta H.L.Wendl. ex Pfeiff.	a. 1a b. Spineless cultivars and selections are not listed.
Pennisetum setaceum (Forssk.) Chiov.	a. 1b b. Sterile cultivars or hybrids are not listed.
Pinus elliotti Engelm. and hybrids, varieties and selections	a. 2 for sterile specimens. b. 1b for non-sterile specimens.
Pyracantha angustifolia (Franch.) C.K.Schneid.	a. 1b b. Sterile cultivars or hybrids are not listed.

SPECIES	CATEGORY / AREA
Pyracantha coccinea M.Roem.	a. 1b b. Sterile cultivars or hybrids are not listed.
Pyracantha crenatoserrata (Hance) Rehder (= *P. fortuneana* misapplied)	a. 1b b. Sterile cultivars or hybrids are not listed.
Pyracantha crenulata (D.Don) M.Roem; including var. *rogersiana* (= *P. rogersiana* (A.B.Jacks.) Chitt.)	a. 1b b. Sterile cultivars or hybrids are not listed.
Pyracantha koidzumii (Hayata) Rehder	a. 1b b. Sterile cultivars or hybrids are not listed.
Vinca major L.	a. 1b b. Sterile cultivars or hybrids are not listed.
Vinca minor L.	a. 1b b. Sterile cultivars or hybrids are not listed.

5